

## RESEARCH ARTICLE

# Formulation and Characterization of Raloxifene loaded Biodegradable Polymeric Nanomicelles

Pooja Dave\*, Chetan Detroja

Department of Pharmaceutics, School of Pharmacy, RK University, Tramba, Rajkot, Gujarat, India

Received: 25<sup>th</sup> December, 2022; Revised: 20<sup>th</sup> January, 2023; Accepted: 23<sup>th</sup> February, 2023; Available Online: 25<sup>th</sup> March, 2023

## ABSTRACT

Raloxifene has been used to treat breast cancer; nevertheless, it has poor water solubility and a low bioavailability fraction because higher first-pass metabolism hinders its application raloxifene nano micelles by using PLGA<sub>50:50</sub> to enhance solubility and bioavailability. The solvent evaporation technique was used to prepare polymeric nano-micelles. Ral-loaded polymeric micelles had a zeta potential of - 0.71 mV, implying a practically neutral surface charge. Blank micelles and RAL-loaded micelles had similar average sizes of 84.29 nm and 95.41 nm, respectively. The drug encapsulating and drug loading capacity was found to be 16.08 ± 0.34% and drug loading 5.04 ± 0.002% (w/w), respectively. An *in-vitro* study illustrates a 95.10% sustained release drug profile for 120 hours.

**Keywords:** Bioavailability, Biodegradability, PLGA50:50, Polymeric Nanomicelles, Raloxifene, Solubility.

International Journal of Drug Delivery Technology (2023); DOI: 10.25258/ijddt.13.1.24

**How to cite this article:** Dave P, Detroja C, Formulation and Characterization of Raloxifene loaded Biodegradable Polymeric Nanomicelles. International Journal of Drug Delivery Technology. 2023;13(1):158-161.

**Source of support:** Nil.

**Conflict of interest:** None

## INTRODUCTION

Breast cancer has surpassed all other female causes of morbidity in recent years. Numerous treatments, including radiation, chemotherapy, immunological therapy, targeted therapy, and hormone therapy, are available for treating breast cancer. In 2007, the Food and Drug Administration (FDA) approved the use of Raloxifene (RAL) to address breast cancer. The FDA originally approved it in 1997 for the treatment of osteoporosis. RAL has been discovered to be an estrogen-receptor inhibitor, reducing the risk of both endometrial and invasive breast cancer. RAL was used to stop estrogen reliance on breast cancer development by interacting through the estrogen receptor in mammary tissues deprived of harming endometrial cells. RAL has a high permeability, but its bioavailability in humans is only 2%, which is extremely low. Due to P-glycoprotein efflux, gut wall glucuronidation, and considerable first-pass metabolism in the liver, RAL also has limited bioavailability.<sup>1</sup>

Nowadays, polymeric micelles have become a promising carrier for poorly water-soluble drugs and also for enhancing kinetic stability. Polymeric Nanomicelles have a spherical shape, resulting from the self-assembly of such a hydrophobic micelle core surrounded by a hydrophilic palisade. Moreover, mixed nanomicelles draw much more attention than single polymeric micelles to enhance stability, low toxicity, high tumor selectivity, penetration, and high drug loading efficiency. Additionally, polymeric nanomicelles contain complex

designs on the size of particles and occurrence to prevent macrophage phagocytosis, hence dramatically increasing the drug's anticancer activity and minimizing its harmful and side effects.<sup>2-9</sup>

Poly lactic-co-glycolic acid<sub>50:50</sub> (PLGA<sub>50:50</sub>) is biodegradable copolymer of an equal ratio of polylactic acid and polyglycolic acid. PLGA<sub>50:50</sub> is the most promising biodegradable and biocompatible polymer, which has a high degradation rate as compared to PLGA<sub>65:35</sub>, PLGA<sub>75:25</sub>, and PLGA<sub>85:15</sub>. RAL-loaded PLGA<sub>50:50</sub> nano-micelles were characterized by various parameters for instance, Particle size, particle size diameter, zeta potential, morphological characterization like, SEM, %drug loading and %entrapment efficiency, *in-vitro* RAL release profile.<sup>10-27</sup>

## METHODS

### Methods and materials

Dr. Reddy's Laboratory generously provided Raloxifene (Hyderabad, India). The acquisition of PLGA<sub>50:50</sub> from Nomisma Healthcare (Vadodara, India). The remaining solvents all came from the analytical grade.

### Formulation and Characterization of Polymeric Nanomicelles

#### Preparation of Blank Micelles

The polymeric nanomicelles were made by a solvent evaporation method. Firstly, triblock copolymer (PLGA<sub>50:50</sub> 10 mg)

\*Author for Correspondence: poojadave.pharma@gmail.com

was dissolved into the acetone, following the mixture was added into deionized water (10 mL) with the help of magnetic stirrers at 2–8°C until a blush tint color clear solution was formed.<sup>24-26</sup>

### Characterization of Polymeric Nanomicelles

#### Fourier Transmittance Infrared Spectroscopy (FTIR)

##### Analysis

Polymeric characterization has been carried out by FTIR analysis using NEXUS 470 FTIR spectroscopy (Nicolet, USA) for functional group analysis.

### Particle size measurement & Zeta potential measurement: Static and Dynamic Light Scattering

A dynamic laser-light scattering instrument measured the particle size and zeta potential of RAL (Zeta Sizer, Parul Institute of Pharmacy and Research, Parul University). Nanomicelles size was determined using a static scattering angle of 90 at 25°C, and the data were estimated using volume distribution. Simultaneously, the zeta potential of nano-micelles was analyzed at 25°C.

### %Drug entrapment efficiency and drug loading efficiency

A UV-visible spectrophotometer set to 287 nm was used to determine the amount of RAL in the micelles. The following formula was utilized to calculate the %drug loading of raloxifene content and %encapsulation efficiency (EE).

$$EE\% = \frac{\text{Weight of Raloxifene in nano - micelles}}{\text{Initial weight of Raloxifene}} \times 100\%$$

$$\text{Drug loading} = \frac{\text{Weight of Raloxifene in nano - micelles}}{\text{Weight of nano - micelles}} \times 100\%$$

Because of raloxifene is poorly water-soluble drug (11 ng/mL), a water content was omitted while comparing %loading capacity of nano-micelles. As a result, free raloxifene in water was not to be considered in the calculation of %DL and %EE.

### In-vitro Dissolution profile

RAL-loaded micellar dispersion equivalent to 1 mg of RAL remained wrapped in a dialysis bag. The sealed bag was suspended in a 20mL methanol: phosphate buffer (7.4 pH) solution. Nano-micellar solution was stirred at 100 RPM at 37°C ± 0.5°C. At specific time intervals, each 1-mL withdrew and substituted with an equivalent volume of fresh diffusion medium. Check absorbance in UV. *In-vitro* drug dissolution study was compared with a standard drug.

### Stability Study

The storage stability was determined by particle size, PDI, and visual inspection of micelles for 15 days.

### Scanning Electron Microscopy (SEM)

SEM determined morphological study of lyophilized micelles.

## RESULTS AND DISCUSSIONS

### FTIR Analysis

Figures 1 and 2 represent the FITR spectra of RAL and PLGA<sub>50:50</sub>. The peaks at 1466.76, 1642.35, and 1245.53 cm<sup>-1</sup> are shown for aromatic rings, keto, and oxo group, respectively,

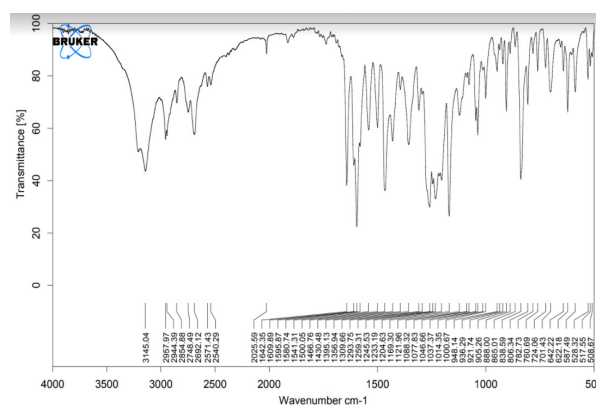


Figure 1: FTIR spectra of ral

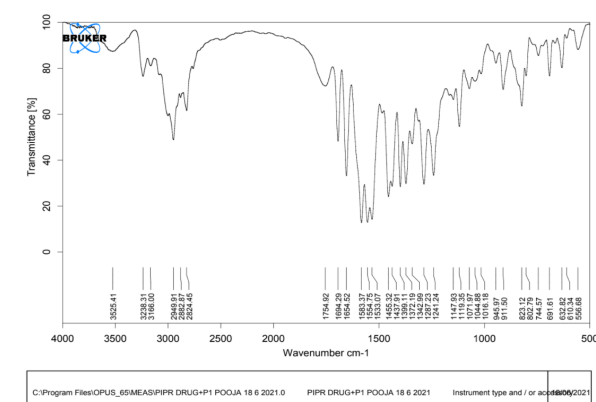


Figure 2: FTIR spectra of plga<sub>50:50</sub>

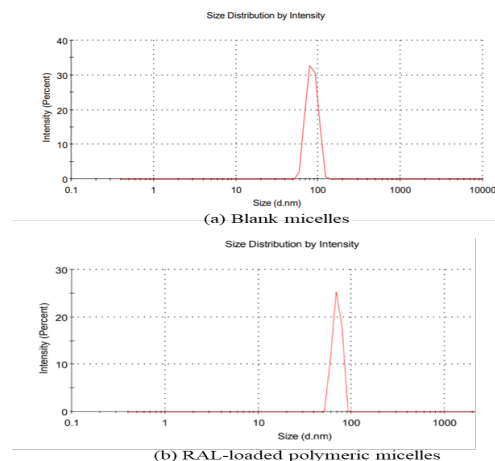


Figure 3: Size of RAL-loaded polymeric micelles

similar to the site of PLGA. The methyl group properties of the polymer were ascribed to the peaks at 1455 as well as 1352 cm<sup>-1</sup>. The properties of the polymer's methylene group placed near the oxygen atom as well as the C-O-C group, were observed to have maxima around 2877 and 1100 cm<sup>-1</sup>.

### Particle Size, PDI, Zeta Potential, and Morphological Characterization

Figure 3 depicts the size distribution of RAL-loaded nanomicelles. RAL-loaded polymeric micelles had a zeta potential of roughly -0.71 mV, implying a practically neutral

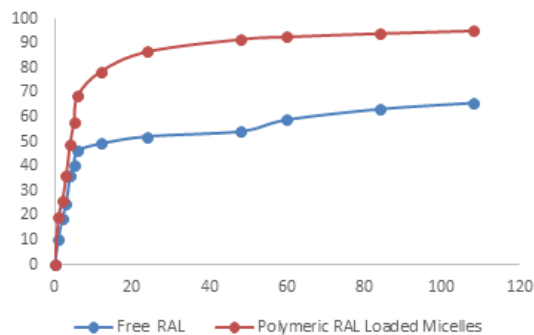


Figure 4: In-vitro drug release study of free RAL and polymeric micelles at pH 7.4

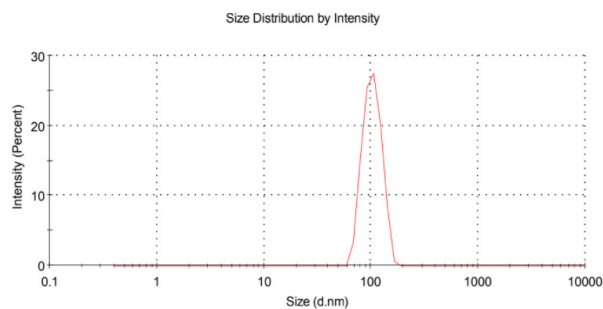


Figure 5: Raloxifene-loaded polymeric Nanomicelles after 15 days

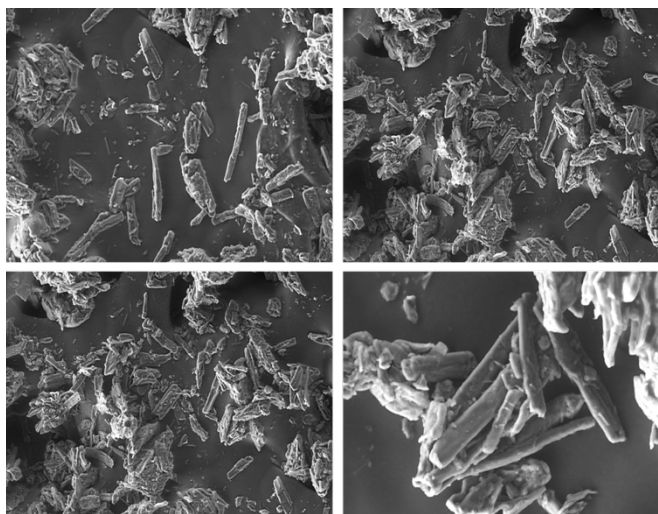


Figure 6: Surface Morphology using SEM

surface charge. Blank micelles and RAL-loaded micelles had similar average sizes of 84.29 and 95.41 nm, respectively, with a tight distribution pattern. The micelles' PDI value was found to be 0.182. Micelles with sizes between 20 and 100 nm have been seen, and they may effectively accumulate by passive diffusion in highly permeable malignancies. When constructing micelles, which represent the features of diffusion over the mucous layer or cellular absorption of epithelial cells, size plays a significant role. In micelles, size and surface charge are important factors in pharmacokinetic measures as well as biodistribution, stability, and diffusivity.

**%Entrapment Efficiency (EE) and %Drug Loading (DL)**

%DL and %EE were found to be  $16.08 \pm 0.34\%$  and drug loading  $5.04 \pm 0.002\%$  (w/w), respectively.

**In-vitro Dissolution Profile**

In the Figure 4, the in-vitro dissolution study, polymeric nanomicelles give a controlled release effect compared with plain drug and enhance raloxifene solubility.

**Storage Stability of Drug-Loaded Micelles**

After 15 days there were small changes in particle size (110 nm) and PDI, but still, it is monodispersed.

**Scanning Electron Microscopy (SEM)**

SEM images of RAL- loaded micelles was shown in Figure 5 PLGA-loaded RAL micelles were spherical in shape and particles in the agglomerated state.

**CONCLUSION**

From the above study, putting in nutshell, Raloxifene loaded PLGA<sub>50:50</sub> micelles were successfully prepared by solvent evaporation method and encapsulated hydrophobic drugs (Raloxifene). Nanomicelles have less size (40–100 nm) due to the smaller size it gives a better absorption and good dynamic stability thus it enhances drug solubility as well as it improves stability of RAL-loaded micelles. These findings suggested that PLGA nanomicelles are highly suitable drug carrier for water-insoluble drugs, potentially improving solubility, biodegradability and bioavailability.<sup>27</sup> Furthermore, from in-vitro dissolution studies it can be concluded that, PLGA<sub>50:50</sub> would help micelles gives controlled release profile for longer period of time and prolong circulation time.<sup>28,29</sup>

**REFERENCES**

1. Ağardan NB, Değim Z, Yılmaz Ş, Altıntaş L, Topal T. The effectiveness of raloxifene-loaded liposomes and cochleates in breast cancer therapy. *AAPS PharmSciTech*. 2016 Aug;17:968-77.
2. Gao ZG, Fain HD, Rapoport N. Controlled and targeted tumor chemotherapy by micellar-encapsulated drug and ultrasound. *Journal of Controlled Release*. 2005 Jan 20;102(1):203-22.
3. Ghosh B, Biswas S. Polymeric micelles in cancer therapy: State of the art. *Journal of Controlled Release*. 2021 Apr 10;332:127-47.
4. Chang X, Wang C, Shan G, Bao Y, Pan P. Thermoresponsivity, micelle structure, and thermal-induced structural transition of an amphiphilic block copolymer tuned by terminal multiple H-bonding units. *Langmuir*. 2020 Jan 9;36(4):956-65.
5. Chen S, Singh J. Controlled release of growth hormone from thermosensitive triblock copolymer systems: in vitro and in vivo evaluation. *International journal of pharmaceutics*. 2008 Mar 20;352(1-2):58-65.
6. Kesharwani SS, Kaur S, Tummala H, Sangamwar AT. Multifunctional approaches utilizing polymeric micelles to circumvent multidrug resistant tumors. *Colloids and Surfaces B: Biointerfaces*. 2019 Jan 1;173:581-90.
7. Perinelli DR, Cespi M, Bonacucina G, Palmieri GF. PEGylated polylactide (PLA) and poly (lactic-co-glycolic acid)(PLGA) copolymers for the design of drug delivery systems. *Journal of pharmaceutical investigation*. 2019 Jul 1;49:443-58.
8. Loh XJ, Chee PL, Owh C. Biodegradable thermogelling polymers. *Small Methods*. 2019 Mar;3(3):1800313.

9. Liu Z, Liu D, Wang L, Zhang J, Zhang N. Docetaxel-loaded pluronic p123 polymeric micelles: in vitro and in vivo evaluation. *International journal of molecular sciences*. 2011 Mar;12(3):1684-96.
10. Luo Y, Yin X, Yin X, Chen A, Zhao L, Zhang G, Liao W, Huang X, Li J, Zhang CY. Dual pH/redox-responsive mixed polymeric micelles for anticancer drug delivery and controlled release. *Pharmaceutics*. 2019 Apr 11;11(4):176.
11. Miller T, van Colen G, Sander B, Golas MM, Uezguen S, Weigandt M, Goepferich A. Drug loading of polymeric micelles. *Pharmaceutical research*. 2013 Feb;30:584-95.
12. Patra A, Satpathy S, Shenoy AK, Bush JA, Kazi M, Hussain MD. Formulation and evaluation of mixed polymeric micelles of quercetin for treatment of breast, ovarian, and multidrug resistant cancers. *International journal of nanomedicine*. 2018;13:2869.
13. Alhakamy NA, Ahmed OA, Fahmy UA, Md S. Development and in vitro evaluation of 2-methoxyestradiol loaded polymeric micelles for enhancing anticancer activities in prostate cancer. *Polymers*. 2021 Mar 13;13(6):884.
14. Ghezzi M, Pescina S, Padula C, Santi P, Del Favero E, Cantù L, Nicoli S. Polymeric micelles in drug delivery: An insight of the techniques for their characterization and assessment in biorelevant conditions. *Journal of Controlled Release*. 2021 Apr 10;332:312-36.
15. Di Cola E, Grillo I, Ristori S. Small angle X-ray and neutron scattering: powerful tools for studying the structure of drug-loaded liposomes. *Pharmaceutics*. 2016 Mar 28;8(2):10.
16. Eshel-Green T, Bianco-Peled H. Mucoadhesive acrylated block copolymers micelles for the delivery of hydrophobic drugs. *Colloids and Surfaces B: Biointerfaces*. 2016 Mar 1;139:42-51.
17. Ambade AV, Savariar EN, Thayumanavan S. Dendrimeric micelles for controlled drug release and targeted delivery. *Molecular pharmaceutics*. 2005 Aug 1;2(4):264-72.
18. Wang S, Xie L, Liu Y, Yang Q, Jia W, Zhao D, Zhao X. Study on the preparation and activity of intelligent response poly (lactic-co-glycolic acid)-ss-polyethylene glycol copolymer micelles. *Journal of Biomaterials Applications*. 2022 Aug;37(2):259-74.
19. Karataş D, Bahadori F, Tekin A, Ergin Kizilcay G, Celik MS. Enhancing the kinetic stability of polymeric nanomicelles (PLGA) using nano-montmorillonite for effective targeting of cancer tumors. *The Journal of Physical Chemistry B*. 2022 Jan 10;126(2):463-79.
20. Perinelli DR, Cespi M, Bonacucina G, Palmieri GF. PEGylated polylactide (PLA) and poly (lactic-co-glycolic acid)(PLGA) copolymers for the design of drug delivery systems. *Journal of pharmaceutical investigation*. 2019 Jul 1;49:443-58.
21. Bahadori F, Büyük AŞ, Kozanoğlu AS, Eskandari Z, Ankaralı H, Kepekçi Tekkeli ŞE, Koçyiğit A. Preparation and evaluation of inflammation targeted nano-micellar formulation of celecoxib. 22. Miller T, van Colen G, Sander B, Golas MM, Uezguen S, Weigandt M, Goepferich A. Drug loading of polymeric micelles. *Pharmaceutical research*. 2013 Feb;30:584-95.
23. Chu B, Shi S, Li X, Hu L, Shi L, Zhang H, Xu Q, Ye L, Lin G, Zhang N, Zhang X. Preparation and evaluation of teniposide-loaded polymeric micelles for breast cancer therapy. *International Journal of Pharmaceutics*. 2016 Nov 20;513(1-2):118-29.
24. Karataş D, Bahadori F, Tekin A, Ergin Kizilcay G, Celik MS. Enhancing the kinetic stability of polymeric nanomicelles (PLGA) using nano-montmorillonite for effective targeting of cancer tumors. *The Journal of Physical Chemistry B*. 2022 Jan 10;126(2):463-79.
25. Wang LL, He DD, Wang SX, Dai YH, Ju JM, Zhao CL. Preparation and evaluation of curcumin-loaded self-assembled micelles. *Drug development and industrial pharmacy*. 2018 Apr 3;44(4):563-9.
26. Perumal S, Atchudan R, Lee W. A review of polymeric micelles and their applications. *Polymers*. 2022 Jun 20;14(12):2510.
27. Wang H, Zhang Y, Liu Y, Ren Y, Wang J, Niu B, Li W. Preparation of curcumin loaded hyaluronic acid-poly (lactic-co-glycolic acid) micelles with pH response and tumor targeting. *European Polymer Journal*. 2022 Aug 15;177:111450.
28. Gothwal A, Khan I, Gupta U. Polymeric micelles: recent advancements in the delivery of anticancer drugs. *Pharmaceutical research*. 2016 Jan;33:18-39.
29. Deng C, Jiang Y, Cheng R, Meng F, Zhong Z. Biodegradable polymeric micelles for targeted and controlled anticancer drug delivery: promises, progress and prospects. *Nano Today*. 2012 Oct 1;7(5):467-80.